The Mutagenic and Carcinogenic Hazards of Complex Polycyclic Aromatic Hydrocarbon (PAH) Mixtures in Contaminated Soils and Other Complex Matrices

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The People Who Did All the Benchwork

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Sanya Petrovic (HC, NCR)
Priority Substance Lists for Human Health Risk Assessment (HHRA)

- Priority substance lists permit pragmatic determination of the risks posed by complex mixtures of pollutants in complex matrices (e.g., soil, drinking water).

- Hazard/risk assessments of complex mixtures assume that total hazard/risk is the sum of the incremental contributions from each prioritised component (for a given mode of action).

- For Polycyclic Aromatic Hydrocarbons, assessments commonly examine risks posed by the list of 16 compounds referred to as the “Priority PAHs”.

Priority Pollutant Lists – Where Did They Come From?

- US Government - As a result of 2 meetings* and a few weeks of data review, 129 substances were listed in the amended Clean Water Act (1976) & these “prioritised” substances became part of US law. The current list of 126 Priority Pollutants can be found in Appendix A to 40 Code of Federal Regulations Part 423.

- At the time (1976), most countries had nothing better, and the list was used as starting point for emerging legislation.

- In Canada - PSL I published in the Canada Gazette in February 1989. 44 substances considered as “toxic” under the Canadian Environmental Protection Act (1988).

- 25 substances added to PSL 2 - Canada Gazette December 1995.

Sources: http://water.epa.gov/scitech/methods/cwa/pollutants.cfm

*See Keith, LH. 2015. PAC 35:147-160.
Priority PAHs (Polycyclic Aromatic Hydrocarbons)
The Relative Potency Approach to Determining the Carcinogenic Risks Posed by Complex PAH Mixtures

“Exposures to mixtures of carcinogenic PAHs should be assessed according to the potency equivalence factor (PEF) scheme ......., carcinogenic PAHs are adjusted to their carcinogenic potency relative to benzo[a]pyrene, and the potency equivalents are then summed.”

<table>
<thead>
<tr>
<th>PAH</th>
<th>PEF Relative to B[a]P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[a]pyrene</td>
<td>1</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>0.1</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>0.1</td>
</tr>
<tr>
<td>Benzo[j]fluoranthene</td>
<td>0.1</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>0.1</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>0.01</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.1</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>1</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Research Question –

Is the carcinogenic activity of PAH-containing complex mixtures equivalent to the incremental sum of the contributions from the known (priority) components?

"...... the genomes of tumour cells are invariably altered at multiple sites, having suffered disruption through lesions as subtle as point mutations, and as obvious as changes in chromosome complement."

Carcinogenesis Involves 6 Essential Pathophysiological Aberrations
Comparison of Two Different Approaches for Determination of BaP Equivalent Concentrations

<table>
<thead>
<tr>
<th>Chemistry-driven Approach</th>
<th>Bioassay-driven Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentrations of target PAHs and PEFs to determine total BaP equiv.</strong></td>
<td><strong>In Vitro (Using Cells)</strong></td>
</tr>
<tr>
<td><img src="image" alt="Chemical structures" /></td>
<td>Responses of mouse cells to determine total BaP equiv.</td>
</tr>
<tr>
<td>[PAHi]xPEFi</td>
<td><img src="image" alt="Green fluorescent cells" /></td>
</tr>
<tr>
<td>[ \sum_{i=1}^{n} [PAHi]xPEFi ]</td>
<td>BaP equivalents (mg/kg) = Potency of PAH-containing mixture</td>
</tr>
<tr>
<td>BaP equivalents (mg/kg) = Potency of BaP</td>
<td>Potency of BaP</td>
</tr>
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</table>

**Bioassay-driven Approach**

**In Vivo (Using Animals)**

Responses of animals (e.g., stomach, intestine, liver, etc) to determine total BaP equivalents

BaP equivalents (mg/kg) = Potency of PAH-containing mixture

Potency of BaP

Effect per unit mixture (e.g., mutations per equiv. kg)

Effect per unit BaP (e.g., mutations per mg)
Research Using Cultured Mouse Cells

Gasworks, Wood Preservation & Coke Oven Sites (i.e., PAHs from high-temperature combustion)
70 – 9000 mg/kg priority PAHs

- Luleå (Coke Oven)
- Holmsund (Wood Treatment)
- Forsmo (Wood Treatment)
- Husarviken (Coal Gasification)
- Hässleholm (Wood Treatment)
Crude Extract

Dry and Homogenize Sample

Accelerated Solvent Extraction

Silica Gel Fractionation
To obtain PAH-containing fraction

Assess ability to induce mutations.
Compare pure PAHs, complex PAH mixtures (soils), & defined synthetic mixtures
Cancer Risk Assessment (Chemical/PEF Method)

Total Excess Lifetime Cancer Risk

\[
\text{Total Excess Lifetime Cancer Risk} = \left( \sum_{i=1}^{n} \left( \frac{C_i \times \text{IR} \times \text{EF} \times 1000}{\text{BW}} \right) \times \text{PEF}_i \right) \times \text{SF}
\]

for PAHs 1 through \( n \)

**IR\(_s\) = 20 mg/day (adult), 100 mg/day (construction worker)**

**EF based on 5 days/week, 48 weeks/year, 35 years of exposure, life expectancy of 75 years**

**AF\(_{GIT}\) = 1**

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<tr>
<td>BaP</td>
<td>1</td>
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<tr>
<td>BaA</td>
<td>0.1</td>
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<tr>
<td>DBahA</td>
<td>1</td>
</tr>
<tr>
<td>B(b)F</td>
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</tr>
<tr>
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<td>B(k)F</td>
<td></td>
</tr>
<tr>
<td>CHRY</td>
<td>0.01</td>
</tr>
<tr>
<td>INDENO</td>
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<td>BghiP</td>
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Cancer Risk Assessment – Bioassay-derived Method

Total Excess Lifetime Cancer Risk

\[
\left[ \left( \frac{\text{Activity}_{\text{soil}}}{\text{Activity}_{\text{BaP}}} \times \text{IR} \times \text{EF} \right) \times \text{BW}^{-1} \right] \times \text{SF}
\]

- Non-targeted, bioassay-derived dose metric.
- Does not require an assumption of additivity.

Excess Lifetime Cancer Risk Posed by PAH-Contaminated Soils – Bioassay-based Method (\textit{in vitro}) and Additive (PEF) Method

Bar Height - calculation using CCME PEFs
Error Bars - difference between lowest and highest of 9 published PEFs

Summary of In Vitro (Cell-based) Results

BaP equivalents in contaminated soils determined using Muta™Mouse in vitro mutagenicity results (bioassay) yield lower excess lifetime cancer risk values relative to those calculated using the traditional additive method (chemistry), but differences are generally small. Metabolic insufficiency?

BOTTOM LINE

Even though the traditional risk assessment methodology is based on few carcinogenic PAHs, for 8 of 10 soils examined, the chemically-determined risk estimates exceed those based on effects measured in cells. Most chemically-derived risk estimates are <5-fold greater than biologically-derived values.
In Vivo Assessment of Mutations Induced by Oral Exposure to Complex PAH Mixtures from Coal Tar (28-day oral)

Muta™Mouse
28-day repeat-dose (oral gavage)

DNA Adducts

Transgene mutations

Micronuclei
Coal Tar *In Vivo* - BaP-Equivalent Concentrations for Risk Assessment (i.e., BaP equiv. per unit coal tar)

No tissue-specific PEFs

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PEF Method: \[ \sum_{i=1}^{n} \text{(Conc. of PAH in mixture)} \times \text{(PEF)} \]

For PAHs 1 through n

Bioassay Method: \[
\frac{\text{Mutagenic Potency of Mixture}}{\text{Mutagenic Potency of BaP}}
\]

Can calculate for each tissue
For remote tissues, PEF method overestimated the BaP-eq. concentration.

For site of contact tissues, PEF method generally underestimated the BaP-equivalent concentration.
Summary of In Vivo (Mouse) Results

BaP equivalents in coal tar determined using Muta™Mouse in vivo results (bioassay), which vary across tissues, suggest that cancer risk estimates determined using the traditional additive approach (chemistry) may be conservative (i.e., high) for remote tissues (e.g., BM) and low for GI tract (site of first contact).

BOTTOM LINE

The traditional risk assessment methodology based on few carcinogenic PAHs provides BaP equivalent values that are largely within 10-fold of values generated using animal bioassay results. For site of contact tissues the values are low, but differences are generally <5-fold. For remote tissues values are high, but also <5-fold.
THE TAKE-HOME MESSAGE

- Not suggesting using a bioassay to routinely assess the level of BaP equivalents in a contaminated soil.
- Use of cell (in vitro) & animal (in vivo) bioassays permitted evaluation of the PEF-based approach to calculate BaP equivalents for cancer risk assessment.
- Animal results show a “port of entry” effect. Chemically determined BaP equivs (and risk estimates) are/will be low for GI tract and high for remote tissues. But differences largely <5-fold.
- PEF-driven calculations under-predict GI tract observations, but differences are small given the magnitude of assumptions, and in line with CCME guidelines. “….. predicted cancer potency of PAH-containing mixtures may deviate from the actual cancer potency …by one order of magnitude or more, deviation may …. be under-predicting ……cancer risks” (CCME, 2010).
- CCME (2010) also indicates “….. a three-fold safety factor should be employed when calculating B[a]P [equivs] for sites affected by creosote or coal tar ….. total B[a]P equivalent should be multiplied by three prior to risk characterization....”
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